

REVIEWS

Role of substance P in inflammatory arthritis

N E Garrett, P I Mapp, S C Cruwys, B L Kidd, D R Blake

The neuropeptide substance P (SP) has wide ranging effects on many cell types. Nilsson *et al* showed that SP could stimulate connective tissue cell growth by stimulating DNA synthesis in human skin fibroblasts and arterial smooth muscle cells *in vitro*.¹ Substance P has also been implicated in inflammation owing to its effects on cells associated with acute and chronic inflammatory responses. Substance P promotes chemotaxis of human monocytes, and this appears to be an SP receptor mediated effect as it is blocked by D-amino acid analogues of SP.² Substance P mediates macrophage activation, including enhancement of the oxidative burst with production of significant amounts of the superoxide anion, hydrogen peroxide, and release of prostaglandin E₂, thromboxane B₂, and leukotriene C₄.^{3,4} A later event induced by SP is the release of lysosomal enzymes from activated macrophages.⁴

Direct injection of SP into human skin produces a wheal and flare reaction as seen with histamine, but SP is about 100 times more potent.⁵ Substance P induces the specific release of histamine from mast cells,⁶ which will produce vasodilatation, oedema, and an increase in vascular permeability—that is, SP can produce an inflammatory response by a direct or an indirect effect. It is apparent that SP may have a role in inflammation and perhaps is involved in the pathogenesis of inflammatory diseases such as rheumatoid arthritis. This review attempts to tie together past and current ideas about SP and inflammatory arthritis.

Substance P

In 1931 Von Euler and Gaddum investigated the distribution of acetylcholine in various organs of the horse.⁷ Extracts from certain tissues, in particular the intestine and the brain, produced a material which caused a lowering of arterial blood pressure in the atropinised rabbit and had a stimulatory effect on the tone and rhythm of atropinised rabbit intestine. Von Euler and Gaddum designated this material as preparation 'P', now known as substance P.

Substance P is an undecapeptide member of the tachykinin family; others include neurokinin A and neurokinin B, which share the common C terminal sequence Phe-X-Gly-Leu-Met (fig 1). There are two distinct genes for mammalian tachykinins: the SP/neurokinin A or preprotachykinin I gene and the neurokinin B or preprotachykinin II gene.⁸ Substance P is encoded by messenger RNAs (mRNA) arising from SP/neurokinin A gene transcription. Although only one gene, there are three mRNAs

(α , β , and γ preprotachykinin) owing to alternate RNA splicing, and all three can encode for SP. However, α preprotachykinin mRNA can only encode for SP, whereas β and γ preprotachykinin mRNA can also encode for neurokinin A and other tachykinins.⁹

Substance P is widely distributed throughout the central and peripheral nervous systems^{10,11} and is synthesised in the cell bodies of peripheral nerves located in the dorsal root ganglia.¹² After synthesis SP is distributed to the central and peripheral nerve terminals by a fast axonal transport system.¹³ The rate of transport to the periphery is greater than the transport rate to the central terminals in the spinal cord.¹⁴

In peripheral nerves SP has been localised to small unmyelinated nerve fibres,¹⁵ described as afferent C fibres, and small myelinated fibres (A delta fibres). These fibres are thought to have a nociceptive function—that is, they are only usually stimulated by painful (noxious) stimuli, with neuropeptides such as SP being the central neurotransmitter.¹⁶ As well as this afferent nociceptive role, SP also has an efferent action, where sensory nerve activation causes antidromic (reversed) transmission of nerve impulses (in addition to orthodromic transmission to the spinal cord) in the branches of the same peripheral C fibre (fig 2). This is known as an axon reflex, and it has been shown that SP is released from sensory nerves after antidromic stimulation.¹⁷ Jansco *et al* showed that antidromic stimulation of a peripheral sensory nerve caused vasodilatation, plasma extravasation into the tissue, and increased vascular permeability,¹⁸ all typical responses seen in acute inflammation. It therefore seems that sensory nerve stimulation results in an acute response, described as neurogenic inflammation, with SP being a possible mediator of the response.

Substance P and joint innervation

For SP to play a part in arthritis there must be sensory nerve innervation to the joint and a role for the nervous system in the pathophysiology of the disease. Clinical evidence for a nervous system involvement came initially from observations by Thompson and Bywaters¹⁹ and Glick,²⁰ who found that in patients whose limbs had been paralysed as a result of an upper motoneurone hemiplegia or a lower motoneurone lesion, such as poliomyelitis, the affected limbs were spared the symptoms of any subsequent development of rheumatoid arthritis (RA). Evidence from animal studies by Courtright and Kuzell showed that sectioning of one sciatic nerve seven days before the induction of

The Inflammation Group,
ARC Building,
The London Hospital
Medical College,
Whitechapel,
London E1 2AD,
United Kingdom
N E Garrett
P I Mapp
S C Cruwys
B L Kidd
D R Blake

Correspondence to:
Professor Blake.

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH ₂
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH ₂

Figure 1 Amino acid sequences of tachykinin peptides.

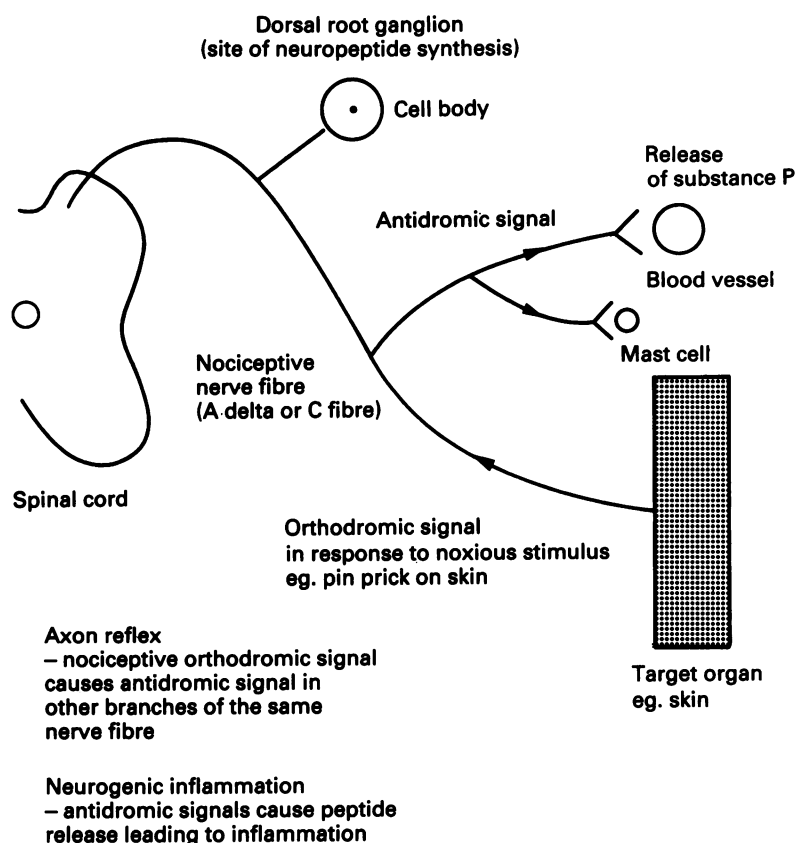


Figure 2 Participation of substance P in inflammation

adjuvant arthritis in the rat delayed the onset and reduced the severity of disease in the operated limb.²¹ Rees *et al* found the opposite, however—that is, denervated limbs developed the disease earlier and more severely.²²

Another indication of nervous system involvement is the exact symmetry of a number of arthritic conditions.²³ Inflammation in one joint is often followed by an inflammatory response in the contralateral joint. A nervous pathway with its precise projection seems a more likely explanation for the observed symmetry than the presence of some blood borne factor. Levine *et al*²⁴ suggested that neural mechanisms may explain three of the clinical features of RA—namely, (a) specific joints at high risk are more likely to develop arthritis; (b) specific joints at high risk have more severe arthritis; and, as stated above, (c) RA is bilaterally symmetrical. It was also suggested in this hypothesis that SP released from peripheral terminals of nociceptive afferent fibres may mediate the neurogenic component of the joint inflammation. Levine *et al* also found that in the adjuvant arthritis model in rats the ankle joints (which are more densely innervated by SP containing neurones than the knee

joints) developed a more severe arthritis than the knee.²⁵ Is there a differential innervation of human joints that reflects the pattern of arthritis in rheumatoid disease?

Using neurofilament antiserum, Gronblad *et al* showed nerve structures in samples of normal, osteoarthritic, and rheumatoid synovium.²⁶ The nerves were mainly perivascular in location, perhaps indicating a regulatory role of the local joint vasculature. When antisera to neuropeptides, including SP, were used immunoreactivity was again shown to be mainly perivascular, but some SP-like immunoreactivity was detected in free stromal nerves—mainly in normal and osteoarthritic synovial samples. As well as a lack of immunoreactivity in the free nerves, the intensity of the staining for neuropeptides was also much weaker in rheumatoid synovia than in normal or osteoarthritic synovia. It is suggested that activation of nociceptors by chemical stimuli—for example, prostaglandins, might have caused an increased release of SP from sensory nerves, thereby lowering the nerve content to below the threshold for detection by immunostaining.

In 1990 Mapp *et al* published a study on the neuropeptide innervation of human synovium, using antiserum to protein gene product 9·5 to map the overall innervation of synovium.²⁷ The advantages of using protein gene product 9·5 antisera are that it is present throughout the neurone and has a cytoplasmic location.²⁸ It also allows the localisation of small diameter fibres (0·5–1·5 μm), such as unmyelinated C fibres. Using this technique, Mapp *et al*²⁷ were able to show a greater number of nerve fibres than had been shown in previous studies in which neurofilament antisera were used. In normal synovium nerve fibres were seen terminating near to the intimal layer of the synovium with some extending to the boundary of the joint space and the intimal layer. The distribution of SP immunoreactive fibres was similar (but less numerous) than protein gene product 9·5 immunoreactive fibres. In rheumatoid synovia protein gene product 9·5 immunoreactive fibres were not found in the synovial intimal layer but had a perivascular innervation of deeper vessels similar to that found in normal tissue. The number of free fibres in deeper tissues was greatly reduced compared with normal synovia, however. In normal tissue SP immunoreactive fibres were most abundant at the synovial membrane with only sparse innervation of synovial blood vessels, and blood vessels and free fibres in deeper tissues. In rheumatoid samples SP immunoreactive fibres were not present in the synovial blood vessels or the synovial membrane and, although present in deeper tissues (either perivascularly or as free fibres), immunoreactivity was much weaker than in normal synovia. As suggested by Gronblad and colleagues, this may be due to increased release of SP from sensory nerves but may be a result of synovial proliferation in joint inflammation outflanking growth of the nerve. Pereira da Silva and Carmo-Fonseca²⁹ confirm the observations of Mapp *et al*²⁷ with the suggestion that rheumatoid synovial tissue has a disturbed neuronal control. The lack of innervation in rheumatoid synovia may be due

to the inactivation or insufficient release of the serine protease inhibitor, protease nexin I. It has been shown that human stromelysin, a connective tissue metalloproteinase released from human synovial fibroblasts in response to inflammatory mediators such as interleukin 1,³⁰ can inactivate the serine protease inhibitor, human α_1 antitrypsin, which shares common structural features with protease nexin I.³¹ Zhang *et al* showed that there was increased proteolytic cleavage of α_1 antitrypsin in knee joint synovial fluid from patients with RA.³² It may be, therefore, that endogenous metalloproteinases inactivate protease nexin I in RA and thus prevent neurite outgrowth, resulting in the absence of nerves to the superficial synovium.

An interesting finding in the study by Mapp²⁷ was the lack of innervation to lymphoid areas in the rheumatoid synovium. Fink and Weihe found neuropeptides, including SP, in nerves supplying the human lymph node.³³ It is known that SP increases proliferation and immunoglobulin synthesis in lymphocytes from murine Peyer's patches, mesenteric lymph nodes, and spleen.³⁴ Substance P reverses the reduced regional lymph node antibody response caused by capsaicin, and it may be that there is a neurogenic control, particularly through nerves containing SP, of normal lymph node function. Thus the lack of innervation and therefore control seen in RA may be responsible for the excessive immunoglobulin production seen in some arthritic patients.

Another possible role for SP and the nervous system in arthritis was suggested by Lotz *et al*.³⁵ They showed that SP was capable of stimulating synoviocytes in RA. It was found that SP increased the release of prostaglandin E₂ from synoviocytes in a manner dependent on dose. It was also shown that SP increased total protein synthesis, including an increase in the synthesis of collagenase. Synoviocyte proliferation induced by SP enhances pannus tissue formation and increases connective tissue damage in the diseased state, thereby identifying a role for SP in the pathology of arthritis.

Concentrations of substance P in joints

Is it possible to detect SP in inflammatory fluids? Tissot *et al* used the carrageenan pleurisy animal model of inflammation and found raised levels of SP-like immunoreactivity in the inflammatory exudate,³⁶ indicating the possible participation of SP in the inflammatory reaction. The first report of SP in human arthritic synovial fluid came from Chapman and Tsao,³⁷ who used high performance liquid chromatography techniques and found SP at a concentration of 0.1 $\mu\text{g/ml}$ in knee joint synovial fluid from an arthritic patient. In 1986 Devillier *et al* reported increased levels of tachykinin-like immunoreactivity in the synovial fluid of patients with rheumatic inflammatory diseases (3.94 (SEM 1.21) ng/ml compared with 1.91 (0.56) ng/ml in non-inflammatory fluid).³⁸

Larsson *et al* using competitive radioimmunoassay found no SP-like immunoreactivity in patients with arthritis or in their control patients.^{39, 40} Larsson and colleagues explained

their findings by an increase in the metabolism of tachykinins in arthritis. This explanation has been re-enforced by evidence from recent work in our laboratory.⁴¹ It has previously been shown that the enzyme neutral endopeptidase (EC 3.4.24.11) can hydrolyse neuropeptides such as SP.⁴² Mapp *et al*,⁴¹ using both a polyclonal and monoclonal primary antibody to neutral endopeptidase and the avidin biotin complex method of detection, did not detect any immunostaining in normal human synovium. In synovial samples from patients with RA, however, there was intense staining of cells surrounding blood vessels and weaker staining in the supporting stroma cells. The increased intensity of staining in cells surrounding blood vessels indicates a vascular origin for the inducing stimulus. It is thought that synovial fibroblasts are the source of neutral endopeptidase. Another study of SP in synovial fluid was made by Marshall *et al*.⁴³ Four clinical groups of patients were studied: those with RA, osteoarthritis, Reiter's syndrome, and arthritis following trauma. It was found that apart from the patients with Reiter's syndrome each group had significantly higher concentrations of SP in synovial fluid than were found in paired plasma samples. Concentrations of SP in the group with RA were 946.6 (SEM 82.8) pg/ml in synovial fluid compared with 676.4 (58.1) pg/ml in plasma. This suggested a localised intra-articular source of SP—namely, release from the peripheral C fibres.

It is apparent from the above mentioned studies that detection of SP in human synovial fluid is extremely difficult. Concentrations reported range from 0 to 0.1 $\mu\text{g/ml}$ (table). The diversity in results may be due to the inaccuracy of the method used (Chapman and Tsao using high performance liquid chromatography found SP concentrations about 100 times greater than those of Marshall) or a lack of sensitivity of the assay (Devillier *et al* used an enzyme immunoassay for tachykinin-like immunoreactivity, not specific for SP immunoreactivity. There is the possibility of cross reactivity with neurokinins A and B and SP fragments). Interpretation of the meaning of the concentrations of any substance in synovial fluid poses a problem because, as yet, the production, degradation, and clearance rates of the system have not been elucidated.⁴⁴

Substance P receptors

If SP has a pathophysiological function in arthritis then it is reasonable to expect altered levels of SP receptors. There are currently three pharmacologically distinct tachykinin receptors—namely, neurokinin 1, 2, and 3. They are defined usually by the rank order of potency of

Published results of substance P (SP) concentrations detected in rheumatoid synovial fluid. Results are given as mean (SEM)

Reference	Substance P (pg/ml)
Chapman and Tsao ³⁷	10 ⁵
Devillier <i>et al</i> ³⁸	3940 (121)
Larsson <i>et al</i> ^{39, 40}	Nil
Marshall <i>et al</i> ⁴³	946.6 (82.8)

tachykinins in binding studies. Substance P has the greatest affinity for the neurokinin 1 receptor but may also interact with neurokinin 2 and 3 receptors, but only at a higher concentration.

It has been shown that the number of receptors for SP/unit area of tissue is increased in inflammatory diseases such as ulcerative colitis.⁴⁵ This is probably owing to an increase in blood vessel density in the tissue rather than an increase in receptor density. Walsh *et al* have recently shown the presence of specific, high affinity, low capacity binding sites for Bolton Hunter labelled ¹²⁵I-SP on endothelial cells of human synovial blood vessels.⁴⁶ Binding characteristics indicated that the receptors were of the neurokinin 1 class. It is known that the binding of SP to its receptor is dramatically increased in the presence of divalent cations, and it may be that changes in physiological concentrations of ions such as Mg²⁺ or Mn²⁺ alter SP binding in vivo (Walsh *et al*, paper presented at European Neuroscience Association, Stockholm, 1990). It has also been shown that there is a large increase in SP receptors in rheumatoid synovium compared with normal synovium.⁴⁷ The receptors were present in greatest concentrations in blood vessels, on endothelial lining cells, and the surrounding smooth muscle cells. Cruwys *et al* showed the presence of a specific vascular receptor for SP in rat synovium,⁴⁸ and it may be that SP participates in the vasoregulation of the synovium, particularly in inflammatory arthritic disease.

The involvement of SP and its receptors in inflammation and arthritis has been studied using a number of animal models. Levine *et al*²⁵ found that infusion of SP into rat knees increased the severity of adjuvant induced arthritis,²⁵ but administration of the SP analogue (d-Pro², d-Trp^{7,9})-SP, a putative SP receptor antagonist,⁴⁹ produced only moderate soft tissue swelling and osteoporosis. Lam and Ferrell showed that the carrageenan model of acute joint inflammation could be virtually abolished by prior administration of the SP antagonist d-Pro⁴, d-Trp^{7,9,10}-SP (4–11).⁵⁰ It has also been shown that injection of capsaicin into the synovial cavity of the rat knee inhibited the inflammation seen following SP injection. Capsaicin causes the release of SP from afferent C fibres and the degranulation of mast cells, but in this case might have caused a depletion of SP receptors in the target tissue.⁵¹ Until recently, antagonists at SP receptors have been analogues of SP itself. Snider *et al*, however, reported that the non-peptide compound CP-96,345 was a potent, reversible, and competitive antagonist of the neurokinin 1 receptor.⁵² Their in vivo rat studies showed that the compound had a lack of agonist activity and was selective in its antagonism. It has also been found that the affinity of CP-96,345 for the neurokinin 1 receptor is dependent on species.^{53–54} The possibility therefore exists of specific neurokinin 1 receptor subtypes for different species.

Andrews *et al* showed that neurogenic plasma extravasation in the rat skin was mediated by the neurokinin 1 receptor.⁵⁵ All this evidence suggests a role for SP receptors in joint inflammation in the rat, and it may be that the use

of specific SP receptor antagonists could have therapeutic value for the treatment of inflammatory joint disease in humans.

Conclusions

This review has concentrated solely on the involvement of substance P, currently the most intensively studied neuropeptide, in arthritic inflammatory disease. It should be noted that SP is one of many neuropeptides (others include calcitonin gene related peptide) which is colocalised with SP in sensory neurones and is also a potent vasodilator.⁵⁶ On the other hand, neuropeptide Y, which is associated with sympathetic neurones and colocalises with the catecholamine synthesising enzymes tyrosine hydroxylase and dopamine-β-hydroxylase,⁵⁷ is a vasoconstrictor. The balance of release of these regulatory peptides is undoubtedly critical.

It is now widely accepted that there is a neurogenic mechanism in the pathophysiology of arthritis and SP is a leading candidate for the role of mediator—it has been found in the required locations, it is present in increased concentration in synovial fluid from rheumatoid patients, and there are altered numbers of SP receptors in rheumatoid tissue. There is also the possibility of a synergistic effect between SP and other neuropeptides, such as calcitonin gene related peptide, to produce the observed inflammatory changes. It is hoped that further research will elucidate the role for neuropeptides in arthritis.

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